PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ZB/2002/642	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/SG02/00091**	International Filing Date *(day/month/year)* 14 May 2002	Priority Date *(day/month/year)* 14 May 2001
International Patent Classification (IPC) or national classification and IPC Int. Cl.[7] C08G 79/04, A61K 47/48, A61P 21/06, 11/06, 9/10, 1/08, 35/00, 11/02, 1/12, 1/10		
Applicant JOHNS HOPKINS SINGAPORE PTE LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **5 sheet(s)**.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand 13 December 2002	Date of completion of the report 21 August 2003
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALBERT S. J. YONG** Telephone No. (02) 6283 2160

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SG02/00091

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-29, 44(abstract)** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **30-34** , received on **14 August 2003** with the letter of **14 August 2003**
- [X] the drawings, pages **45-49** , as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [X] The amendments have resulted in the cancellation of:

- [ ] the description, pages
- [X] the claims, pages 35-43
- [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SG02/00091

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-27	YES
	Claims	NO
Inventive step (IS)	Claims 1-27	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-27	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

CITATIONS

D1. US 5194581
D2. US 5952451
D3. US 6166173
D4. WO 98/46286
D5. WO 98/48859
D6. WO 99/00446
D7. WO 00/19976
D8. WO 00/57852

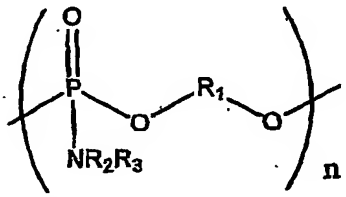
NOVELTY (N) AND INVENTIVE STEP (IS)

Claims 1-27: The claimed invention relates to a positively charged biodegradable polyphosphoramidate that is capable of forming a complex with negatively charged bioactive macromolecules.

The closest art, D1, discloses a biodegradable poly(phosphoester) whereby a therapeutic agent capable of being released in a physiological environment is covalently attached to polymer backbone as a pendant group or forms part of the backbone itself. The citation does not teach the formation of complexes. Hence, the claims are novel and inventive.

What is claimed is:

1. A water soluble and positively charged biodegradable polyphosphoramidate that is capable of forming a complex with negatively charged bioactive macromolecules in aqueous solutions and comprises the recurring monomeric unit shown in Formula I,



FORMULA I

wherein

R_1 is a divalent aliphatic organic moiety;

R_2 and R_3 are each independently selected from the group consisting of hydrogen, alkyl, or heteroalicyclic groups;

each non-hydrogen occurrence of R_2 and R_3 is substituted with one or more positively charged groups; and

n is from 20 to 2,000.

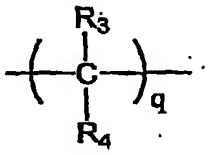
2. A positively charged biodegradable polyphosphoramidate of claim 1, wherein the biodegradable polyphosphoramidate has between about 20 and about 2,000 phosphoramidate groups.

3. A positively charged biodegradable polyphosphoramidate of claim 1, wherein non-hydrogen occurrences R_2 and R_3 are substituted with one or more charged groups selected from the group consisting of primary amine, secondary amine, tertiary amine, quaternary amine or imidazoyl.

4. A positively charged biodegradable polyphosphoramidate of claim 1, wherein one or more of R_1, R_2 or R_3 is substituted with one or more groups capable of facilitating intracellular delivery of a negatively charged bioactive macromolecules, selected from the group consisting of lysosomalytic agent, an amphiphilic peptide, or a steroid derivative.

5. A positively charged biodegradable polyphosphoramidate of claim 4, wherein the group capable of facilitating intracellular delivery of negatively charged bioactive macromolecules is a cholesteryl group.

6. A positively charged biodegradable polyphosphoramidate of claim 1, wherein R_1 is defined in Formula II,



FORMULA II

wherein

each occurrence of R_3 and R_4 are independently selected from the group consisting of hydrogen or alkyl group; and

q is 2 to 4.

7. A positively charged biodegradable polyphosphoramidate composition formed by complexation in aqueous solutions comprising:

(a) at least one negatively charged bioactive macromolecule; and

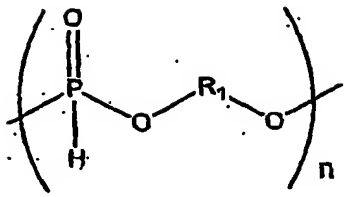
(b) a water soluble and positively charged biodegradable polyphosphoramidate of claim 1.

8. A positively charged biodegradable polyphosphoramidate composition of claim 7, wherein the negatively charged bioactive macromolecules are selected from the group consisting of DNA, RNA, proteins, and polysaccharides.

9. A positively charged biodegradable polyphosphoramidate composition of any one of claims 7 and 8, wherein the biodegradable polyphosphoramidate is capable of complexing 20-60% by weight of the negatively charged biomacromolecules.

10. A method of preparing a water soluble and positively chargeable biodegradable polyphosphoramidate of Formula I, comprising the steps of:

(a). reacting a precursor polymer with recurring unit shown in Formula III,



FORMULA III

wherein

R_1 is a divalent aliphatic organic moiety;

with a primary or secondary amine having a structure of HNR_2R_3, wherein each occurrence of R_2 and R_3 are selected from the group consisting of hydrogen or positively charged alkyl or heteroalicyclic containing protected primary amine, protected secondary amine, tertiary amine, and quaternary amine; followed by

(b). deprotecting the protected amino groups, if applicable.

11. A method of preparing a positively charged biodegradable polyphosphoramidate of claim 10, wherein the biodegradable polyphosphoramidate has between about 20 and about 200 phosphoramidate groups.

12. A method of preparing a positively charged biodegradable polyphosphoramidate composition of claim 7, comprising the steps of:

mixing an aqueous solution of the positively charged biodegradable polymer of Formula I with concentrations ranging from 1 μg/ml to 500 μg/ml,

with an aqueous solution of one or more biological active macromolecules, which is able to complex with polymer of Formula I.

13. A method of preparing a positively charged biodegradable polyphosphoramidate composition of claim 12, wherein the negatively charged or bioactive macromolecules are selected from the group consisting of DNA, RNA, proteins, and polysaccharides.

14. A method of preparing a positively charged biodegradable polyphosphoramidate composition of claim 12 or 13, wherein the biodegradable

polyphosphoramidate is capable of complexing 20-60% by weight of the negatively charged bioactive macromolecules.

15. A method of preparing a positively charged biodegradable polyphosphoramidate composition of claim 12 or 13, wherein the biodegradable polyphosphoramidate has between about 20 and about 200 phosphoramidate groups.

16. A method for the controlled release of a bioactive macromolecule comprising the steps of:

providing a positively charged biodegradable polyphosphoramidate composition of claim 7, and

contacting the composition in vivo or in vitro with a biological fluid, cell or tissue under conditions conducive to the delivery of at least a portion of the biologically active substance to the biological fluid, cell or tissue so that the biologically active substance is released in a controlled manner.

17. A method of claim 16, wherein the bioactive macromolecule is released in-vivo.

18. A method of claim 16, wherein the bioactive macromolecule is released in-vitro.

19. A method of claim 16, wherein the bioactive macromolecule is released extracellularly.

20. A method of claim 16, wherein the bioactive macromolecule is released intracellularly.

21. A method of claim 16, wherein the bioactive macromolecule(s) are selected from the group consisting of DNA, RNA, proteins, and polysaccharides.

22. A method of claim 16, wherein the biodegradable polymer is capable of complexing 20-60% by weight of the negatively charged bioactive macromolecule.

23. A method of claim 16, wherein the biodegradable polymer has between about 20 and about 200 phosphate groups.

24. A method of claim 16, wherein the bioactive macromolecule is a growth factor.

25. A method of claim 16, wherein the bioactive macromolecule is selected from the group consisting of DNA sequences, genes, gene fragments, DNA encoding vaccines, therapeutic agents, cytokines, immunoadjuvants, cancer therapeutic agents, proteins, and combinations thereof.

26. A method of claim 25, wherein the DNA sequence, gene or gene fragment is administered in connection with gene therapy.

27. A method of any one of claims 17 through 26 wherein the positively charged biodegradable polyphosphoramidate composition, including complexes or nanoparticles is delivered in vivo.